

Multiscale Patterned Biosurfaces

Eric J. Amis

Collaborators

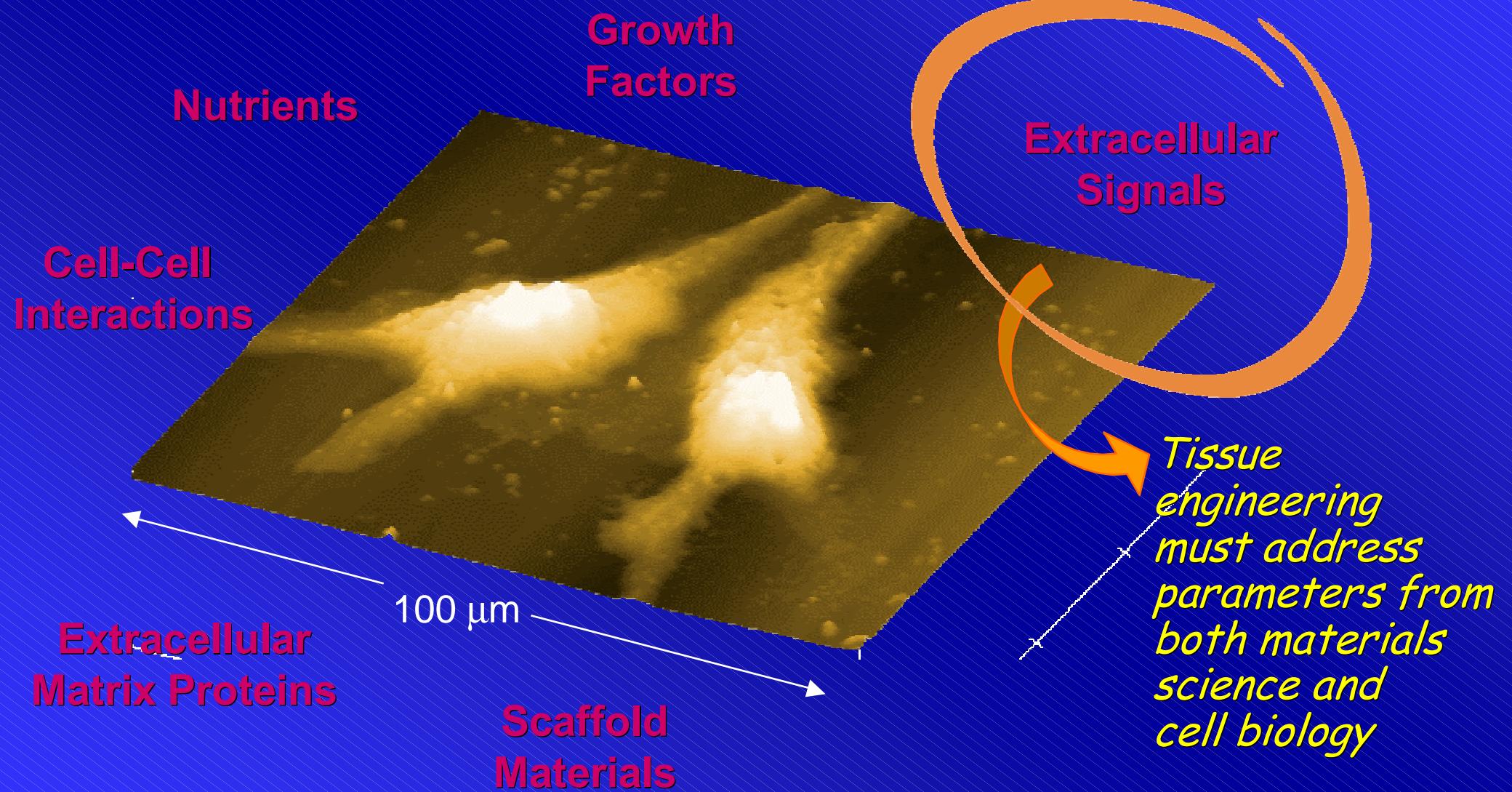
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Materials Science and Engineering Laboratory*

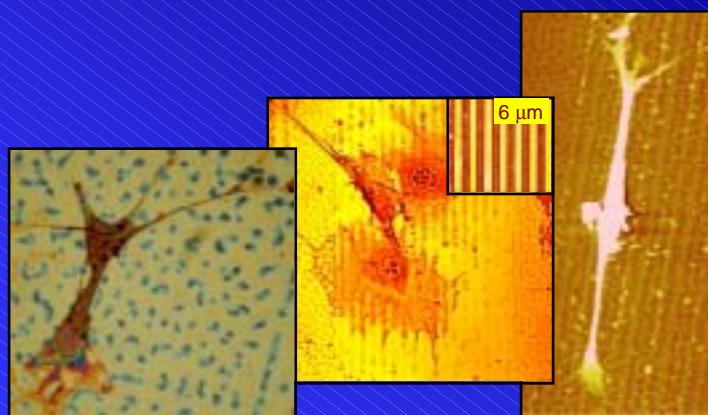
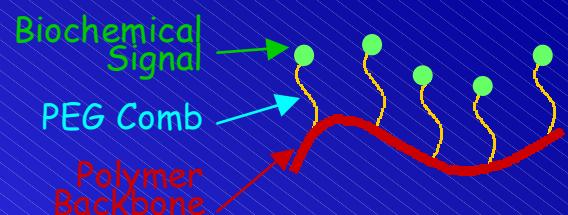
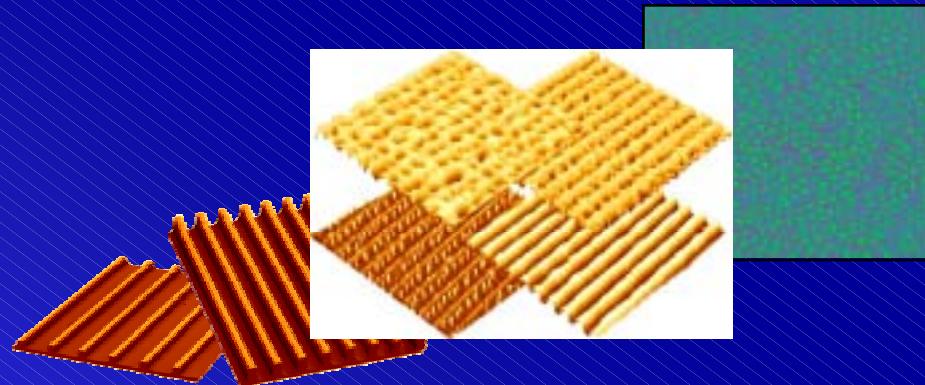


National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

Complex Interactions at Bio/Material Interface



Reference Materials that Guide Cellular Response



Biological materials have structures controlled on scales from millimeters to nanometers. Biomimetic materials require control of:

- Microstructure and nanostructure
- Physical properties and biochemical signals

Designing materials to guide cellular response

Polymer blends: Biomaterial candidates

Traditional

Poly(L-lactic Acid)

Drug delivery

Poly(glycolic acid)

Sutures

Polyurethanes

Artificial valves

Polyethylene

Hip Prostheses

Blends – Novel multicomponent biofunctional systems

- Bioinspired (eg. Bone, cartilage)
- Combine structural and functional components
- Morphological Control

Thermodynamics
Patterning
Processing

*Large Parameter Space \Rightarrow Combinatorial
and High-Throughput Approach*

Phase Separation

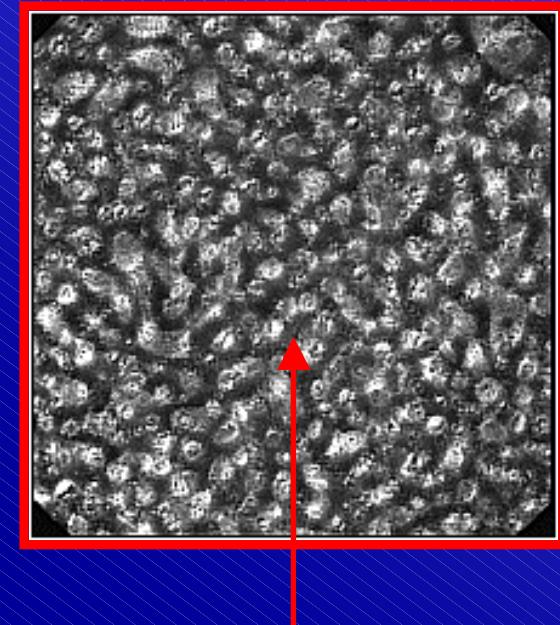
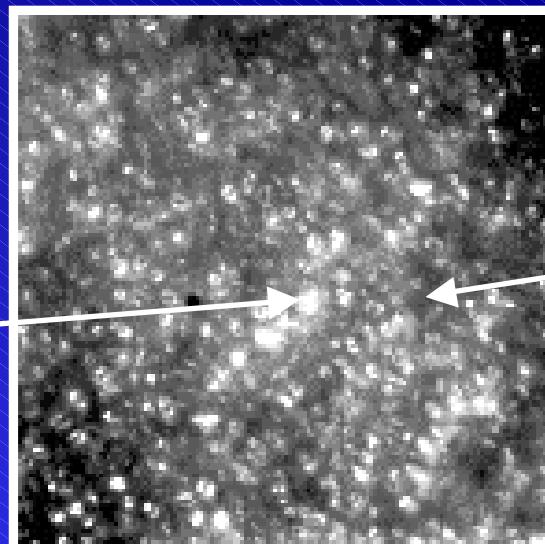
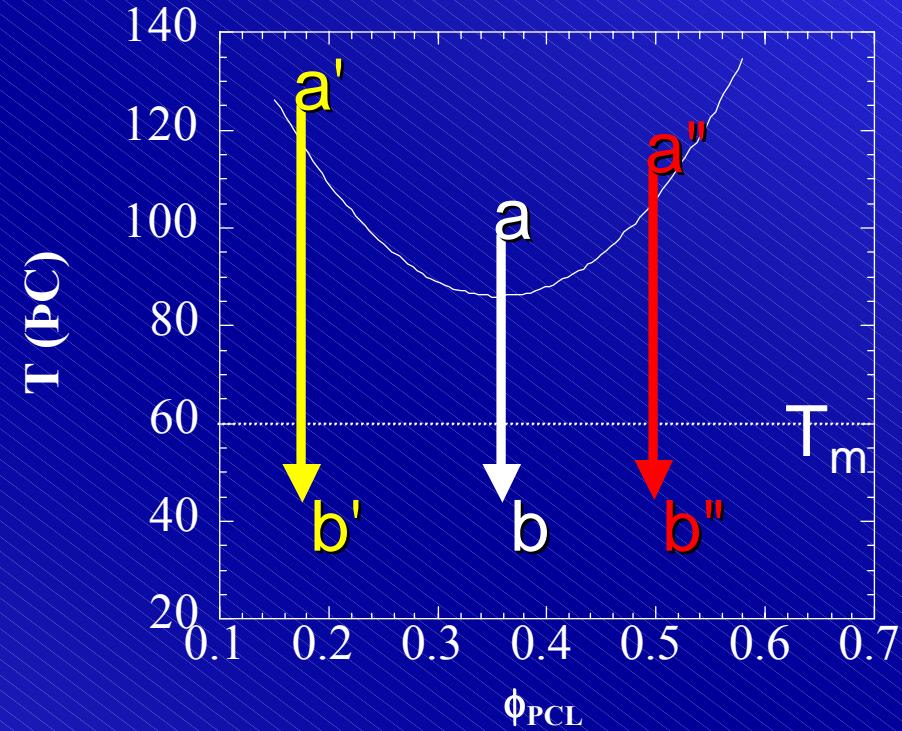
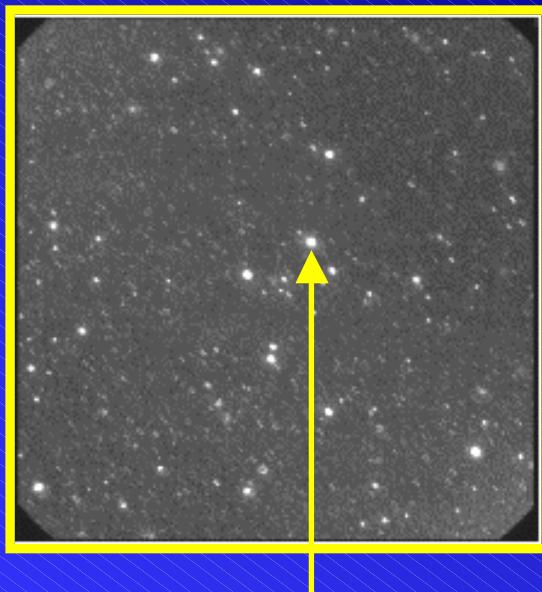
Hot

Cold



Polymer Blend Microstructure

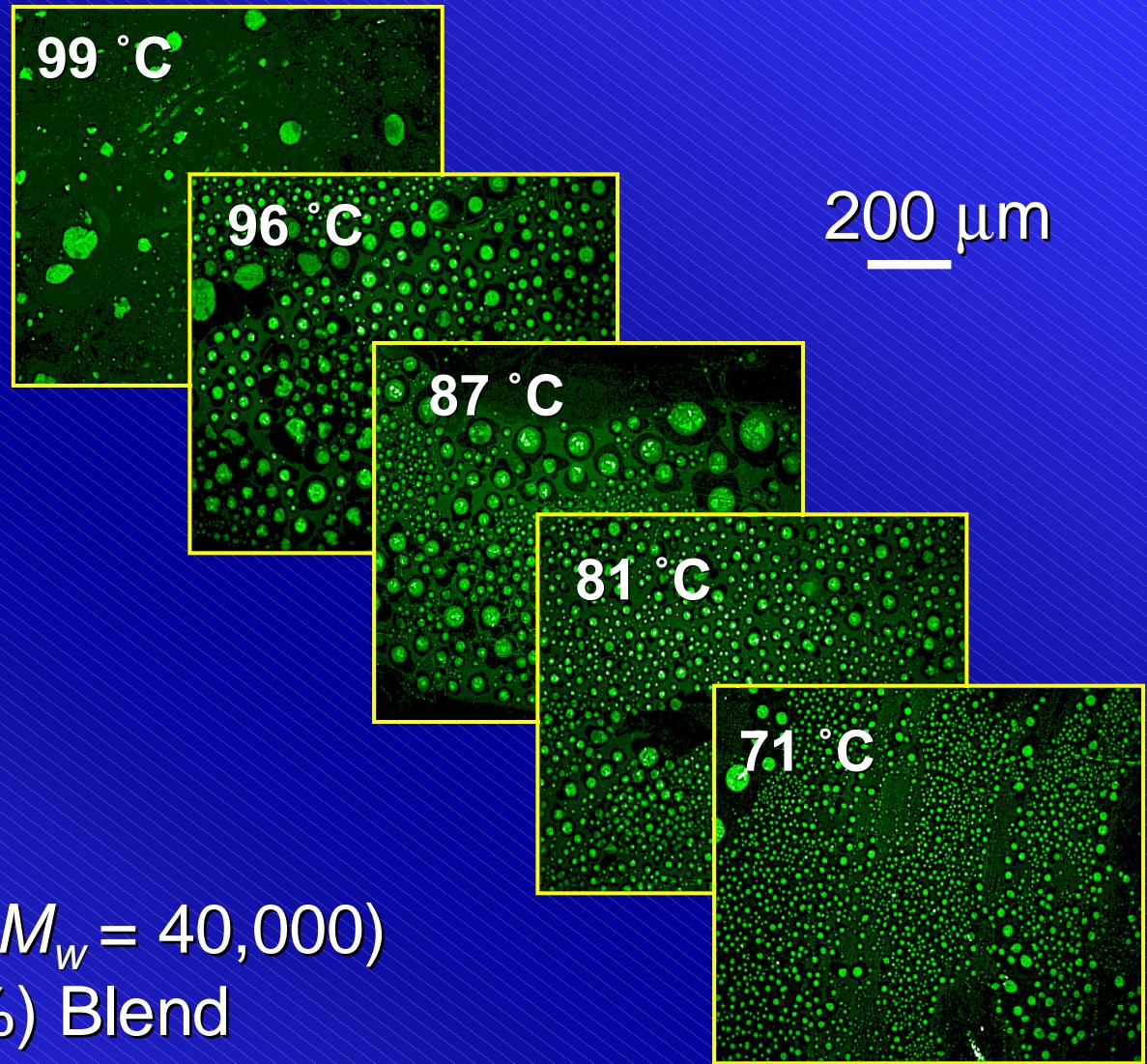
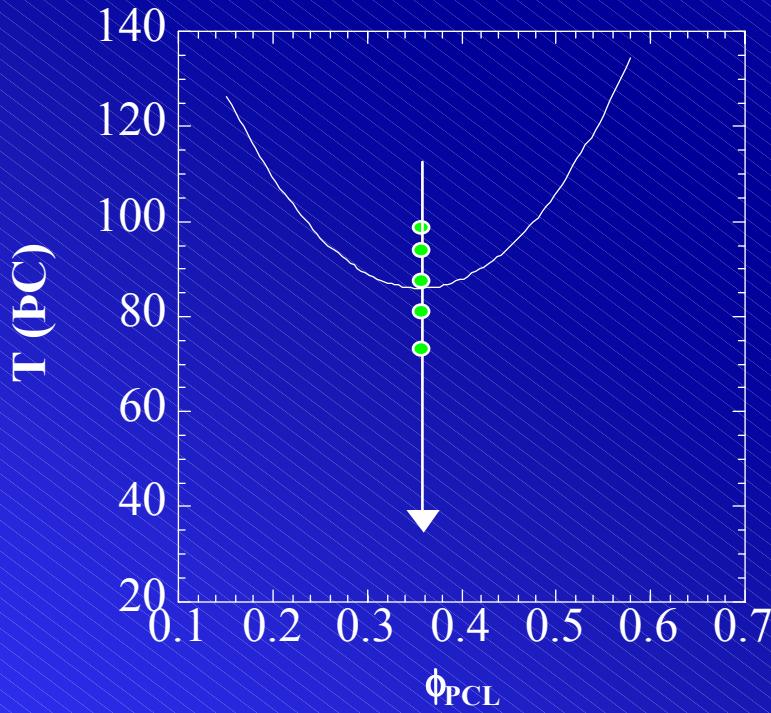
Blend of:
Poly(lactide) with
poly(caprolactone)



PDLLA rich

Meredith and Amis, *Macromol. Chem. Phys.*, **201**, 733 (2000)

Protein Absorption on Two Phase Blend

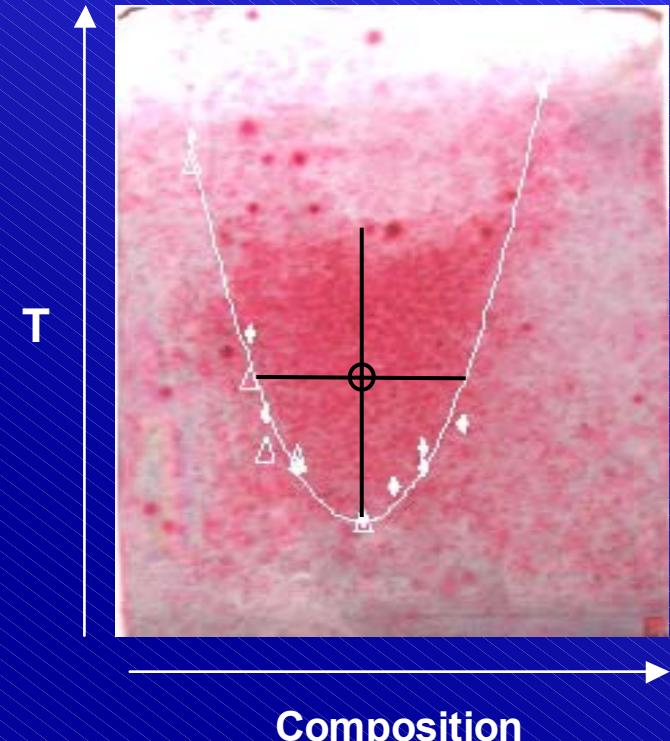


FITC-labeled protein A ($M_w = 40,000$)
PCL (36%) / PDLLA (64%) Blend

High-Throughput Investigations of Cell-Material Interactions

- The massive parameter space inherent in tissue engineering makes the development of combinatorial methods appealing.
- Gradient libraries may form the basis for advanced assays of biomaterial safety and efficacy, both in product development and quality control.

Spanning complex parameter spaces in single experiments

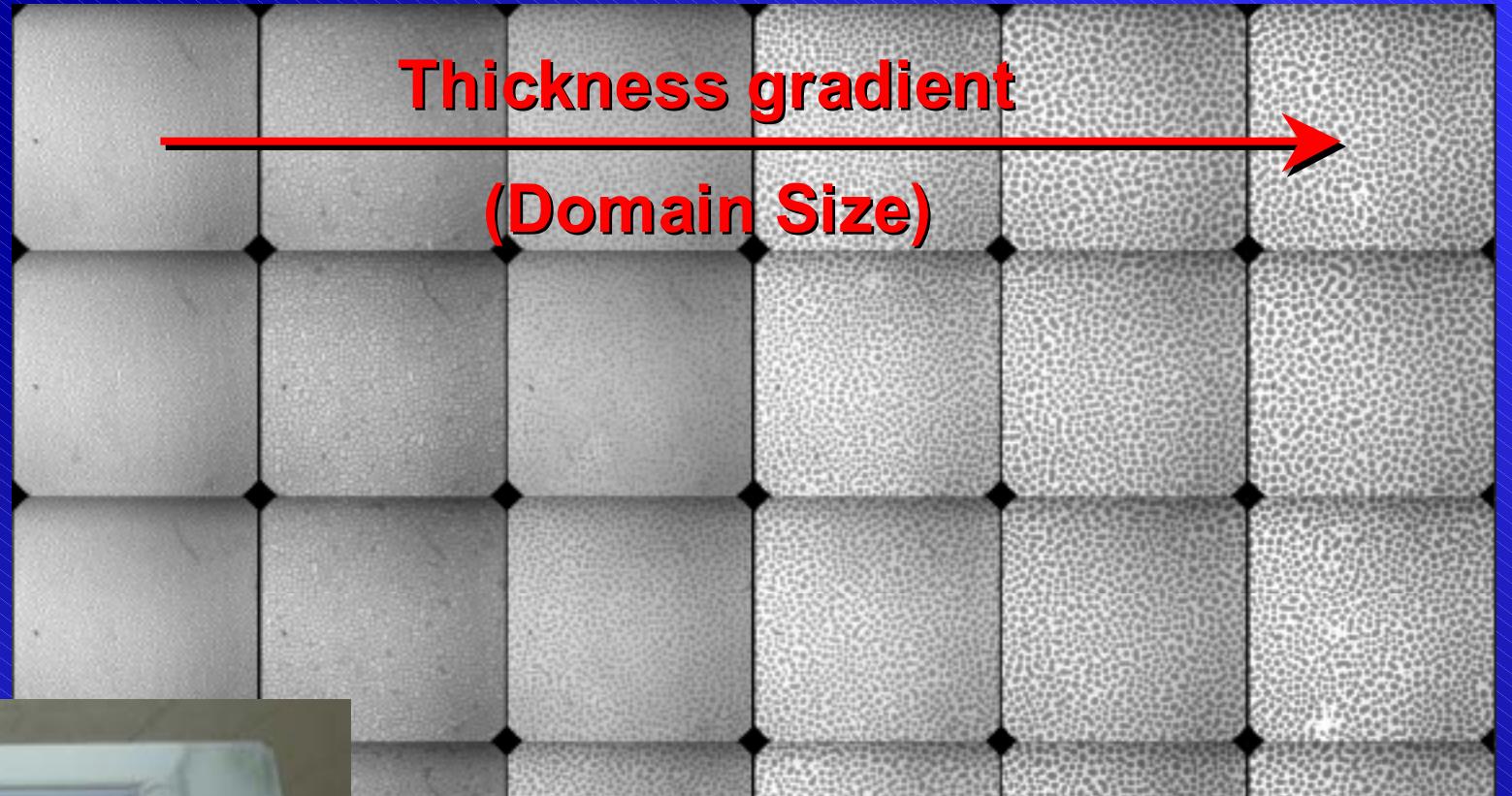


The interaction of cells with polyester blends shows an unexpected response as a function of blend composition and processing temperature

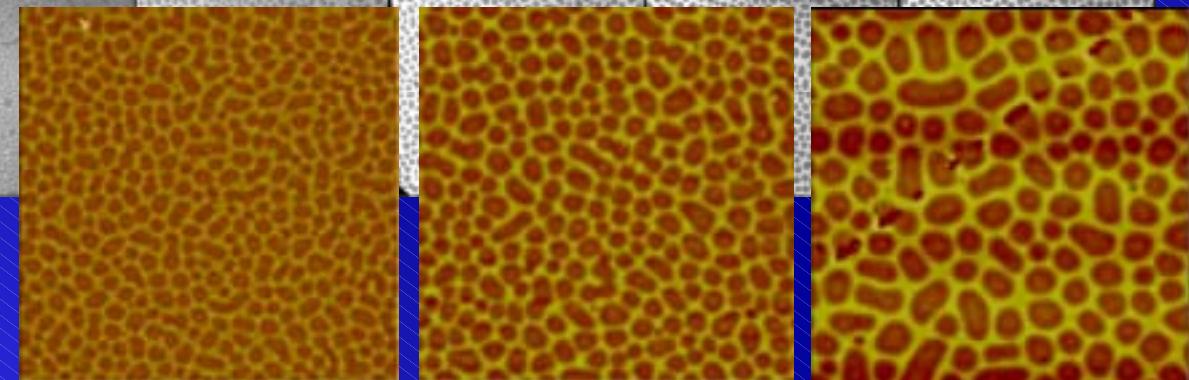
Image courtesy of Dr. J. Carson Meredith (MSEL)

Thickness Gradient PCL / PLA Phase Separated Blend

Optical Microscopy

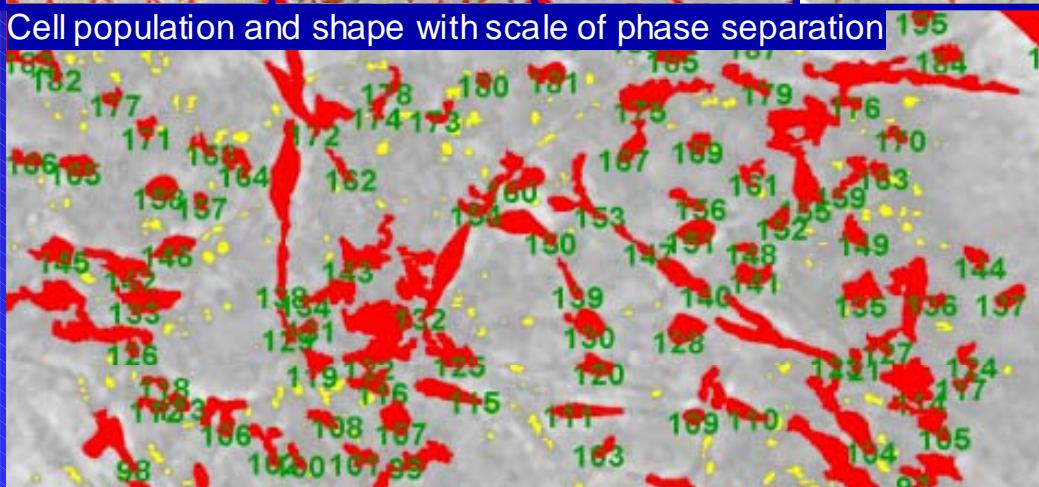
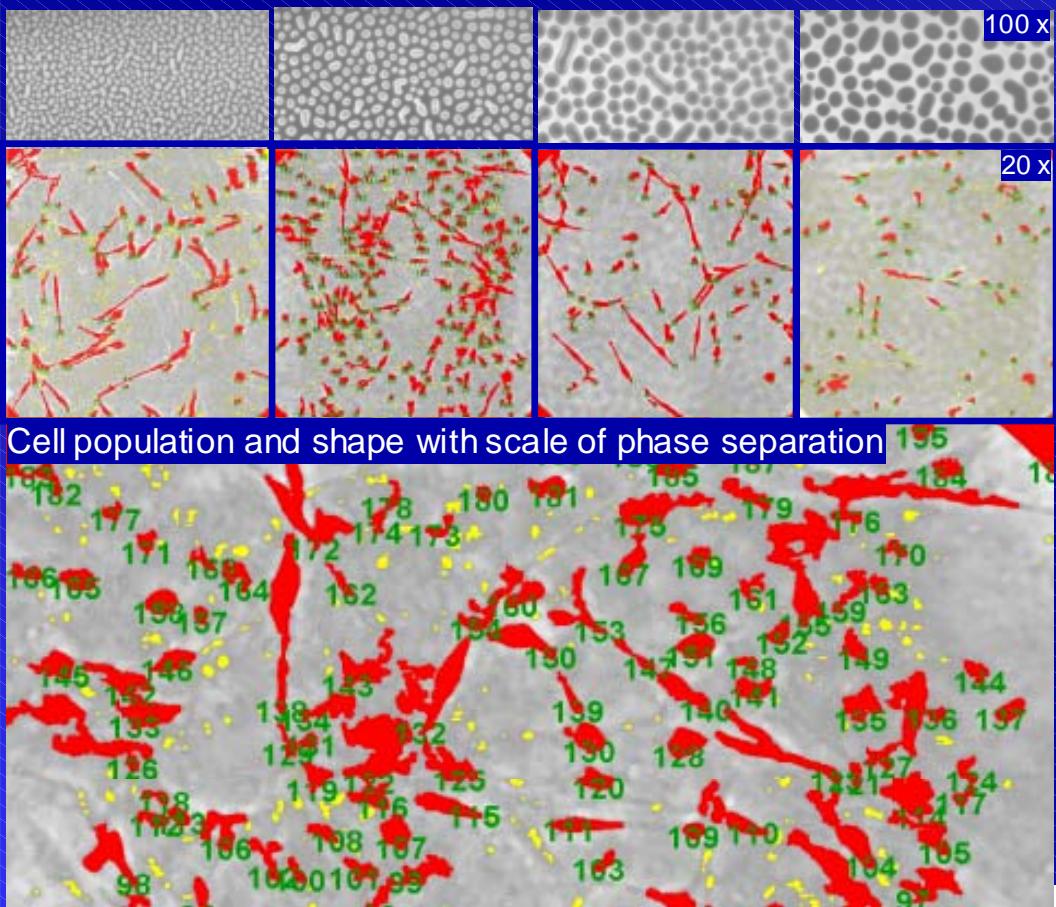


As-cast film



Atomic Force Microscopy

Screening for Optimal Scales of Surface Structure



Day 1- 20x (750 μ m)

Variation of surface property



Cell response

Composition

Adhesion

Lateral Scale (ξ)

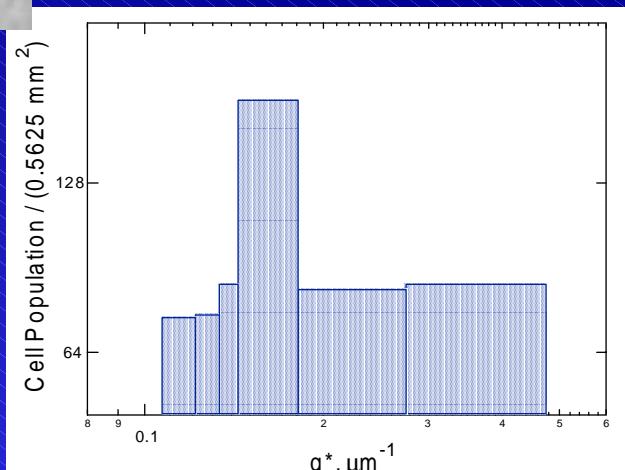
Spreading

Roughness (ζ)

Shape

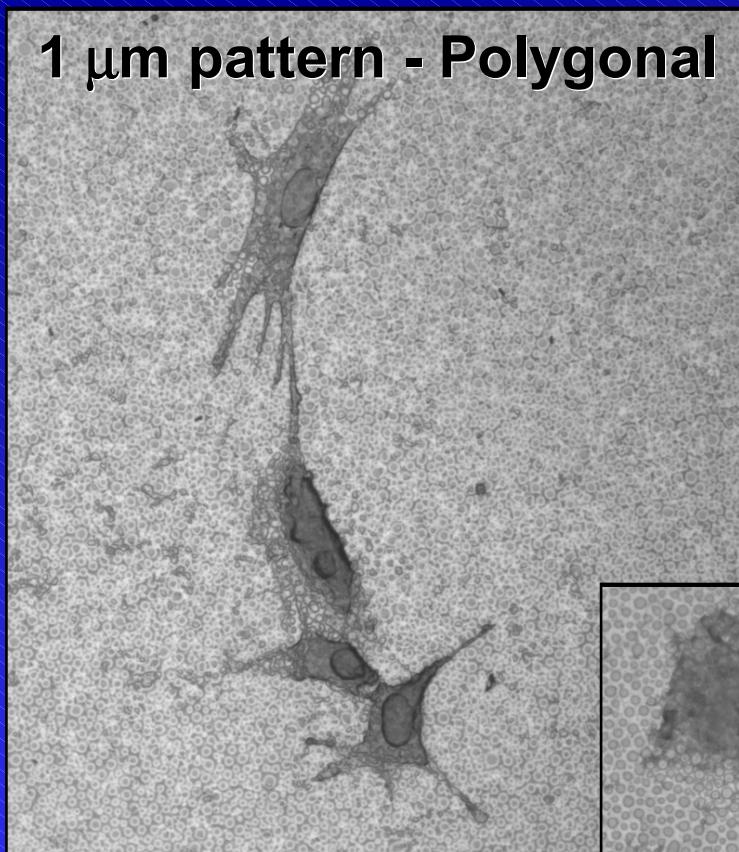
Crystallinity

Proliferation

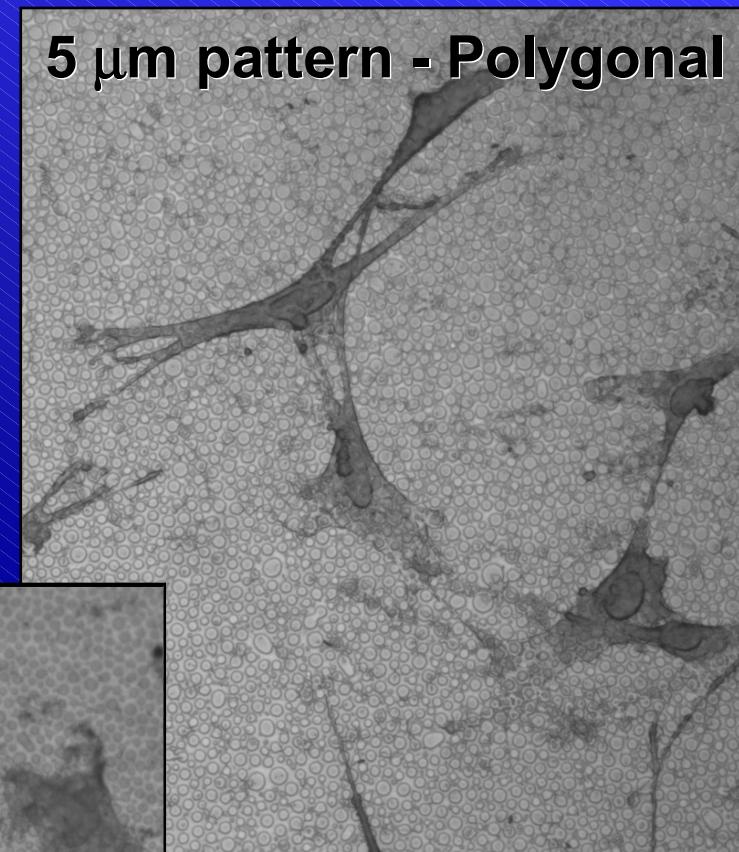


Cell Morphology Control with Topography

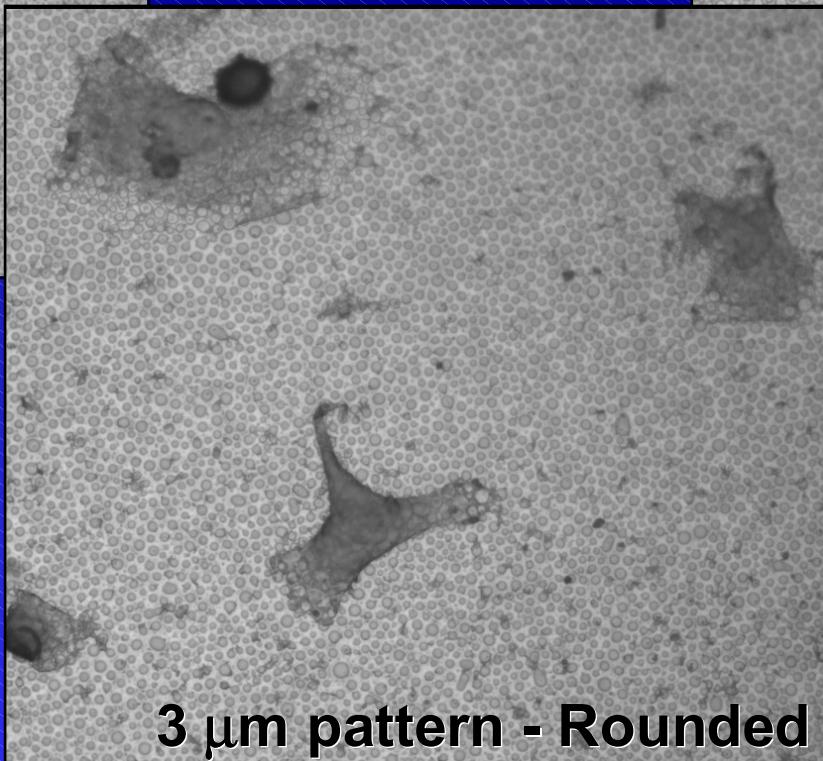
1 μm pattern - Polygonal



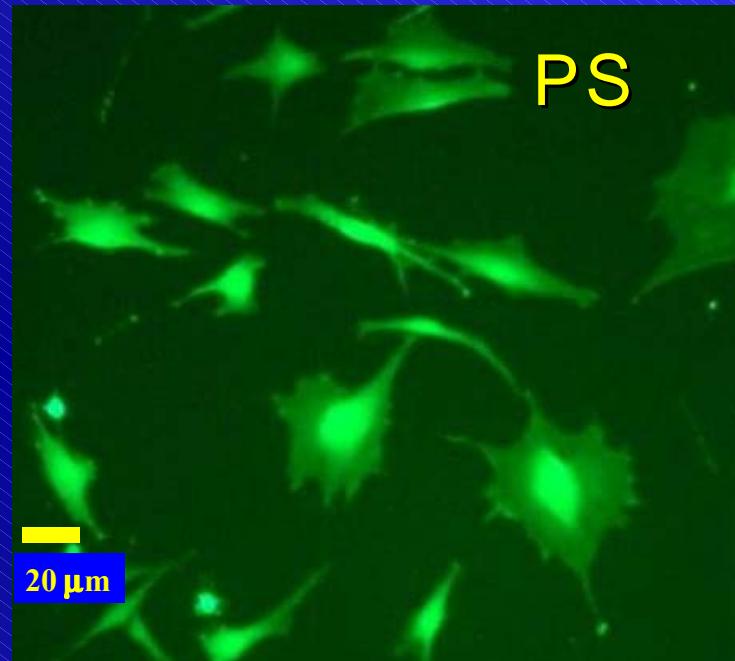
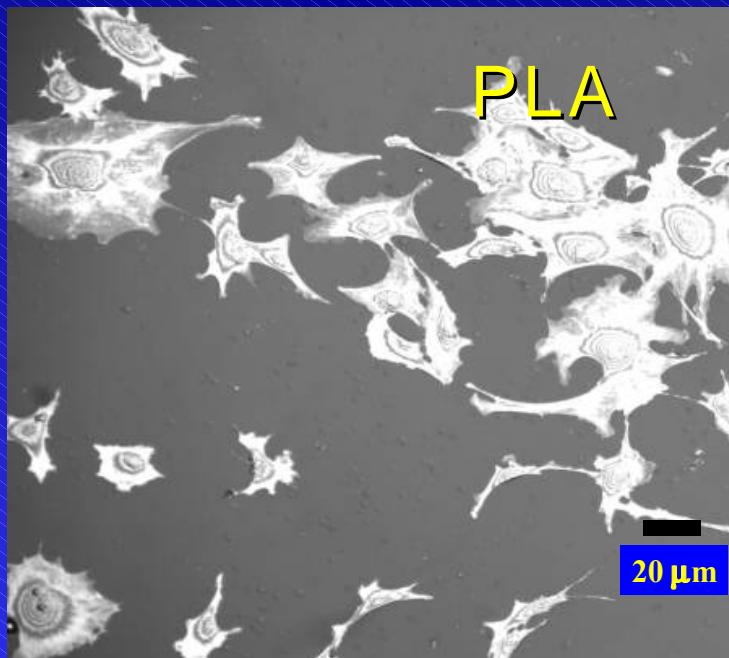
5 μm pattern - Polygonal



3 μm pattern - Rounded



Osteoblastic (MC3T3-E1) Cells on PLA and cultured PS controls (3 Days)



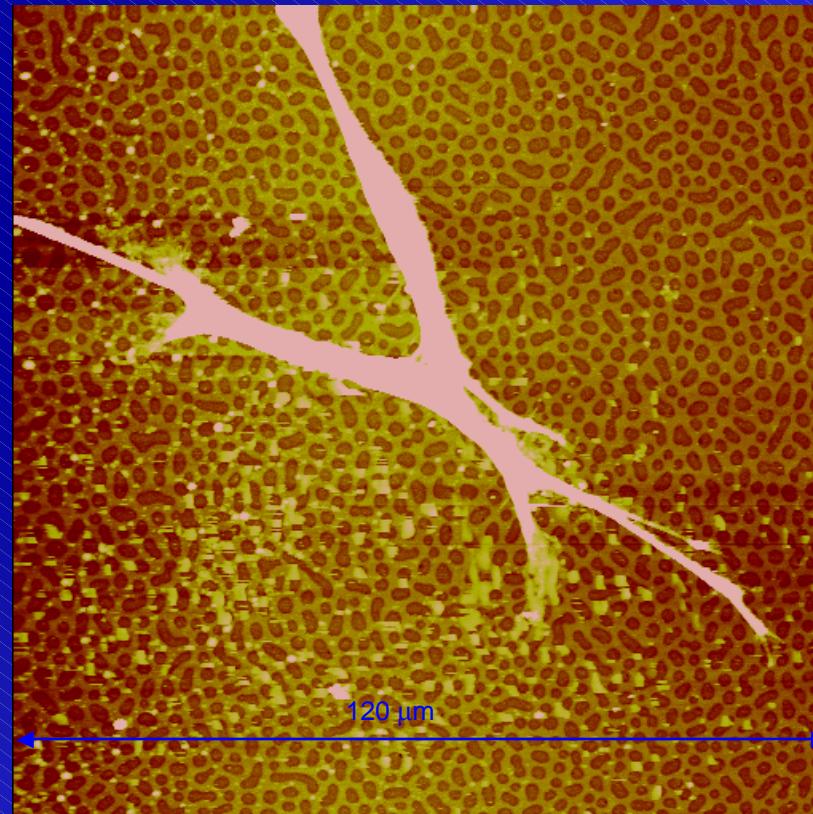
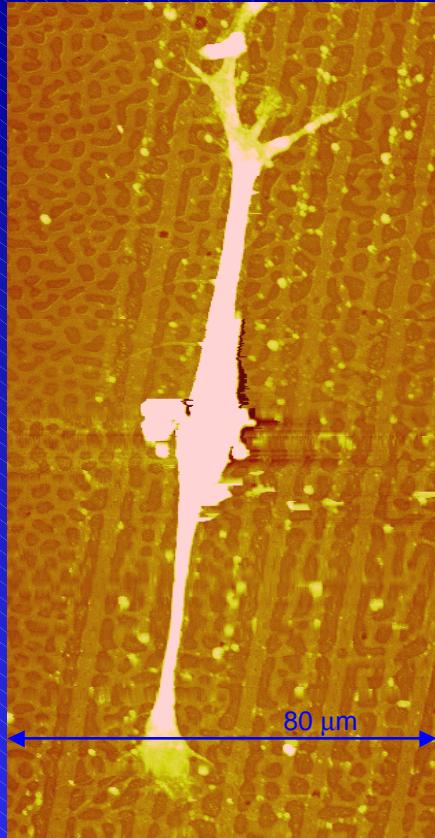
Day 1 – Osteoblast Cellular Response

Libraries of controlled spatial distribution of bio-reception

Pattern Directed vs. Isotropic Region Cell Adhesion

Anisotropic
cell adhesion

Specific focal
adhesion
(PDLA)

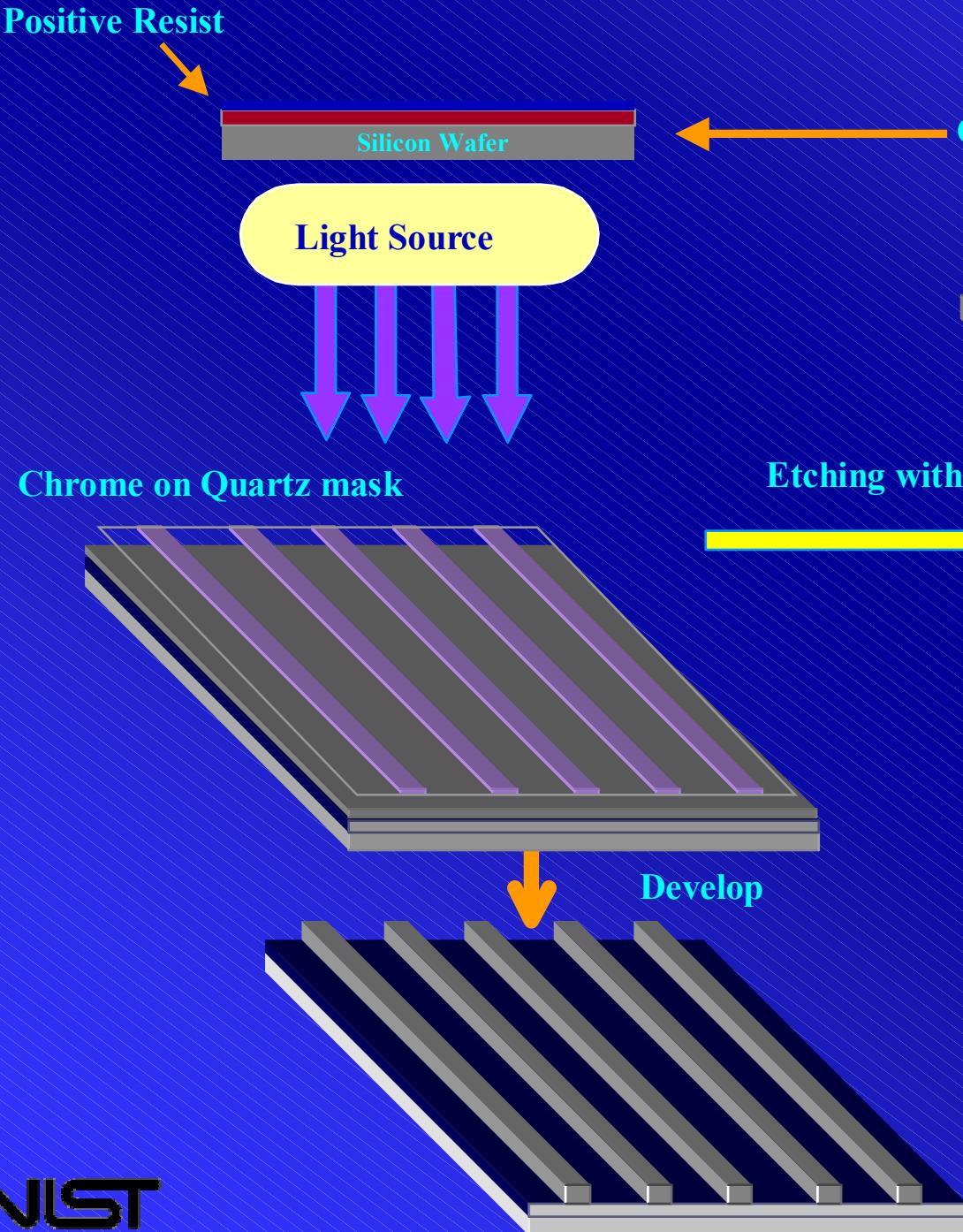


Isotropic cell
adhesion

Specific focal
adhesion
(PDLA)

AFM Images

Lithographic Pattern Production



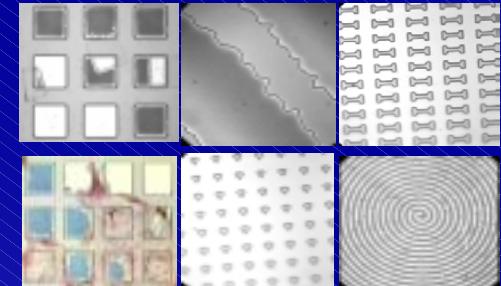
Oxide Layer (1 μm thick)

Etching with HF

Chrome on Quartz mask

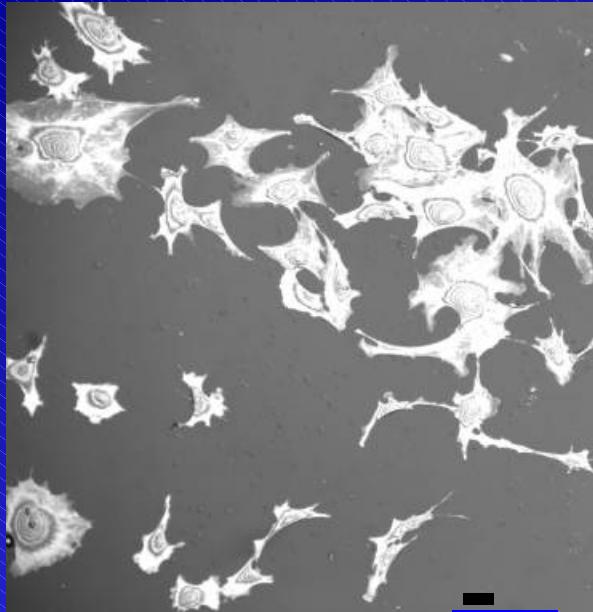
Stamps for
Chemical Patterning

Physical Patterning

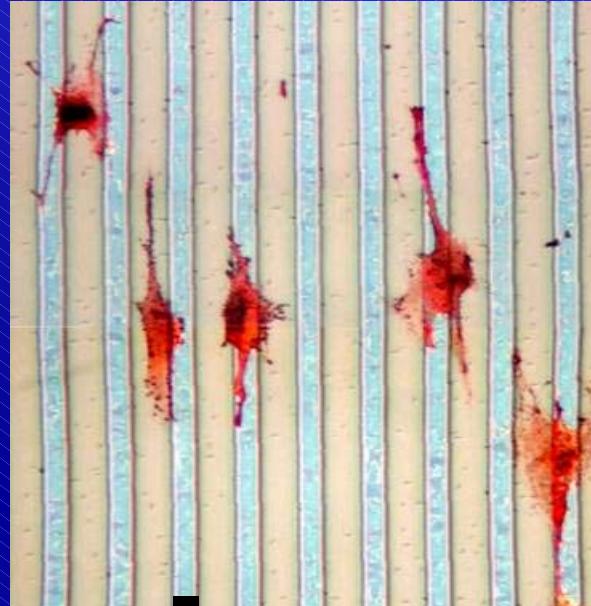


A range of patterns from 2 to 100 μm .

Cells Alignment on Grooves and Ridges (12 μm) How Is Directed Cell Growth Accomplished?



PLA
Spreading Area $\sim 2100 \text{ }\mu\text{m}^2$



PLA coated grooves
Spreading Area $\sim 900 \text{ }\mu\text{m}^2$

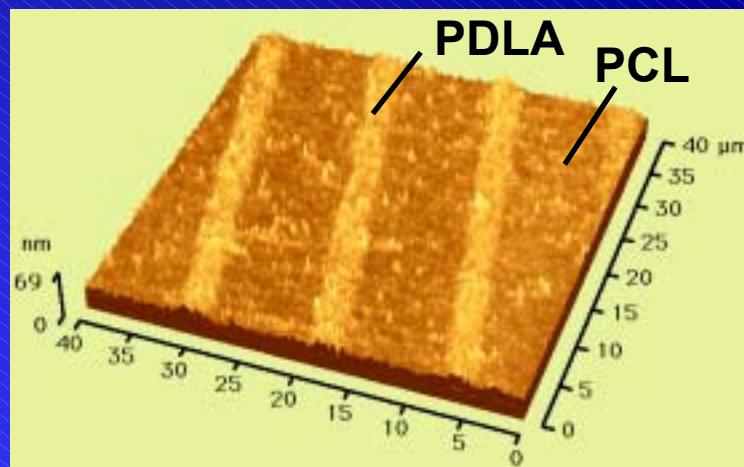
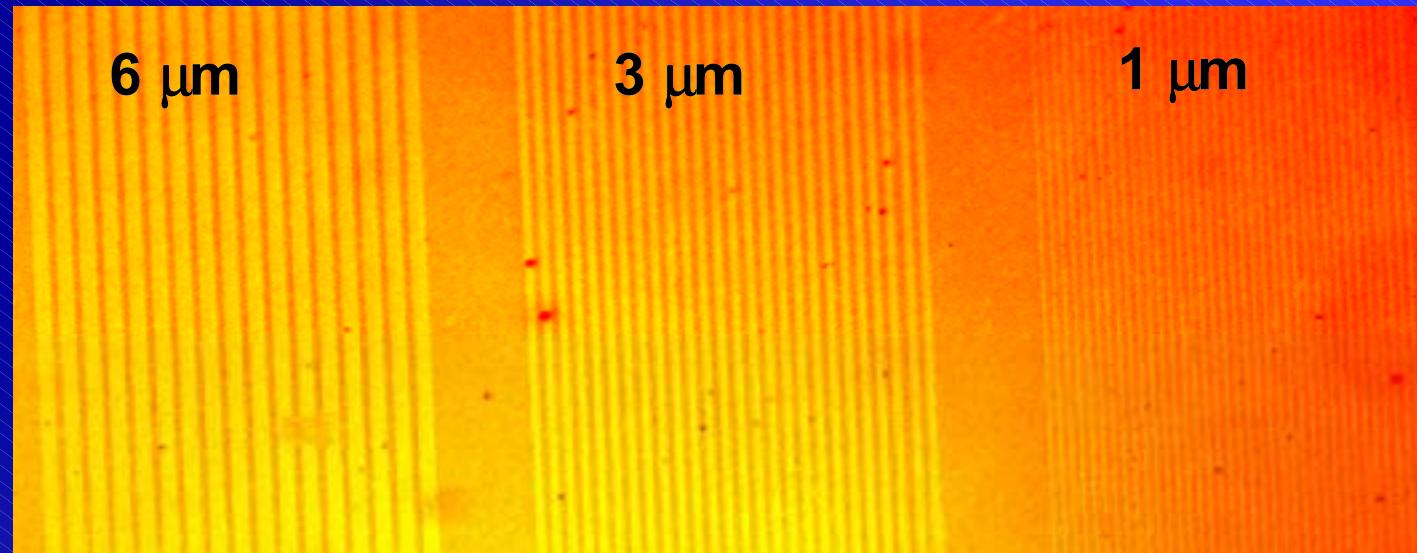
“Ridge Walking”

Cells Sense Topography By Reacting to Discontinuities
Cells are able to bridge smaller grooves
Deeper Grooves Cause Confinement Effects

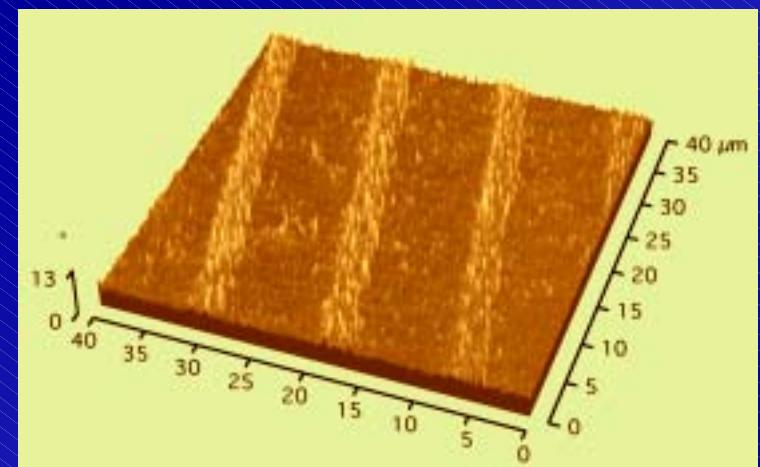
Pattern Directed Phase Separation

Libraries of spatial distribution of bio-reception

Poly- ϵ -caprolactone
and
Poly-D-lactic acid
(36/64)



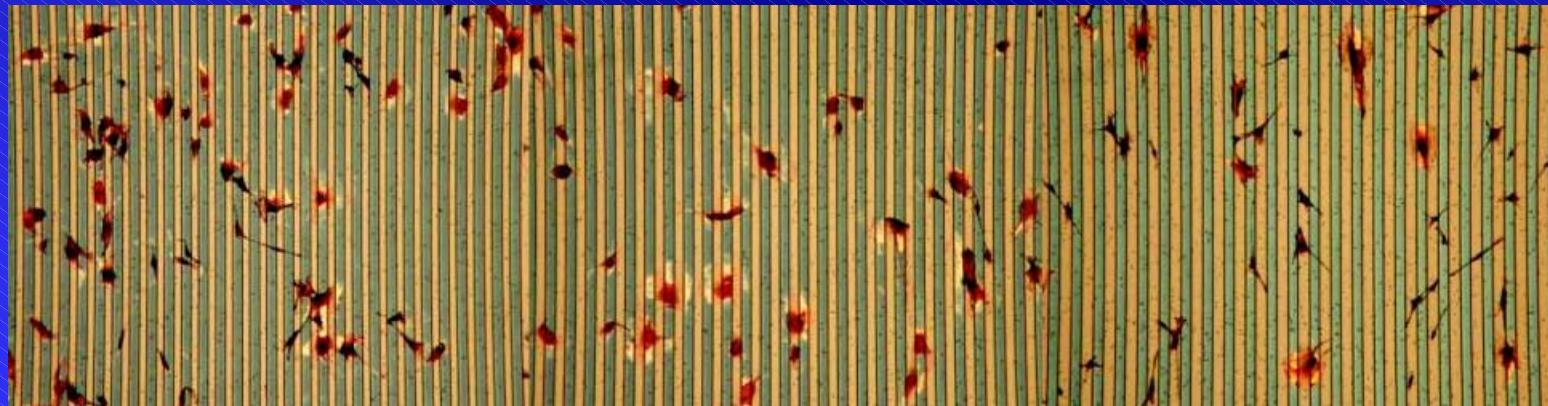
Topographical Image



Phase Image

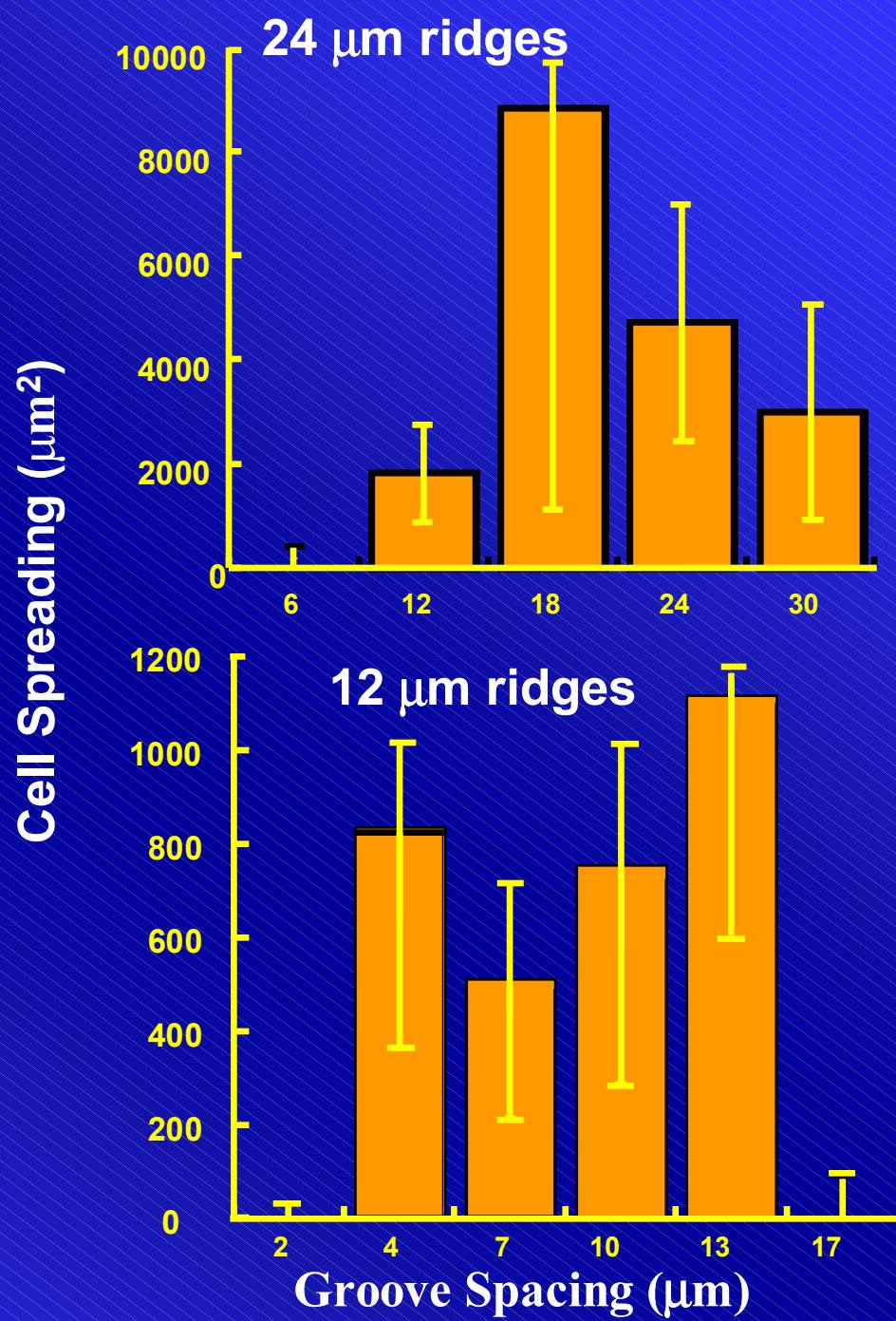
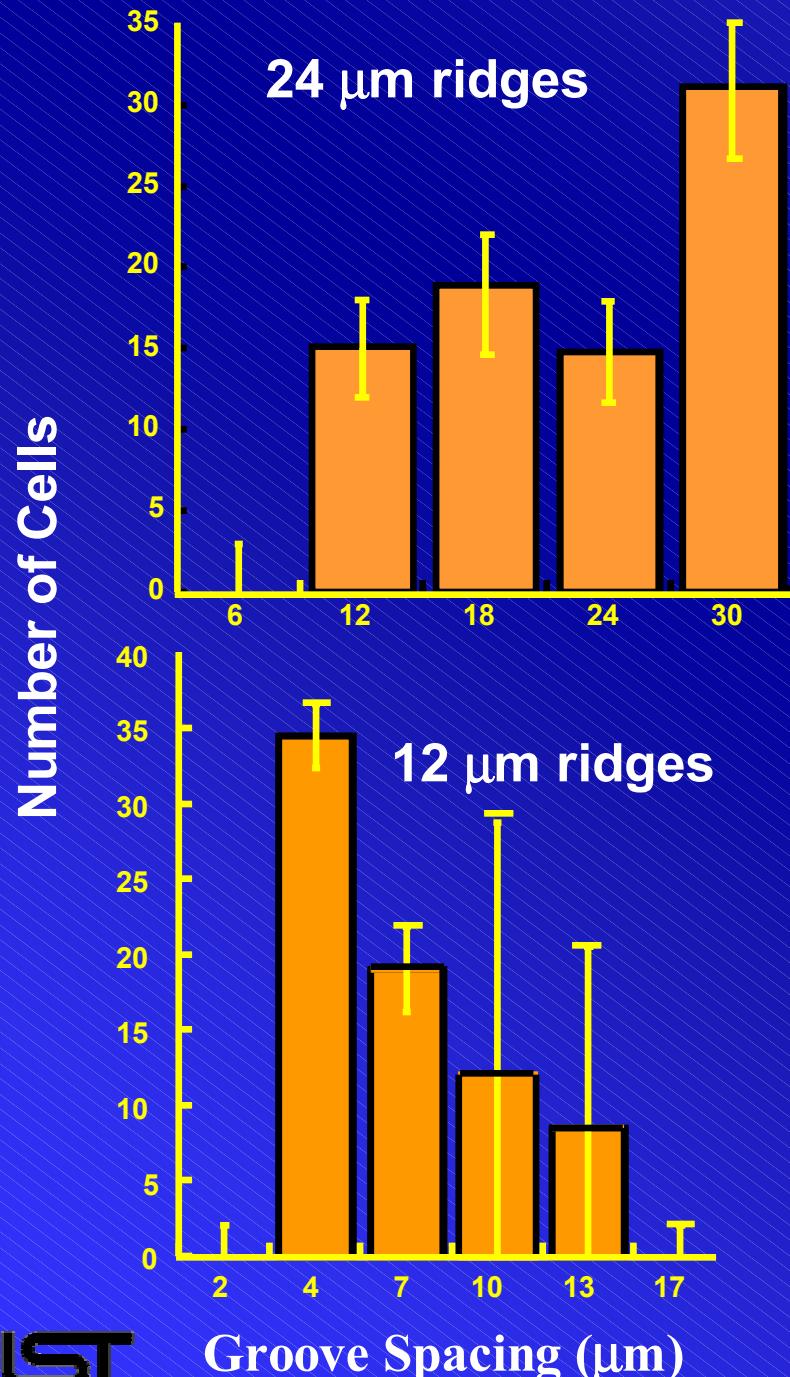
**Osteoblastic cells on PLA Coated Stripes
Groove Width varying from 2-21 μm (3 Days)**

← 4 μm → ← 6 μm → ← 12 μm →



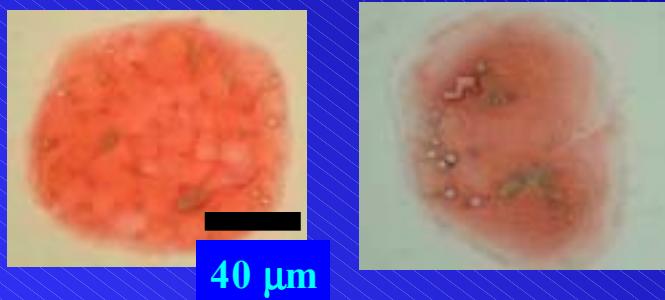
← 18 μm → ← 24 μm → ← 30 μm →

Cell Reaction to Grooved Topography



Epithelial Cells on Stripes (3 days)

- ✓ Epithelial Cells are smaller and proliferate faster than the osteoblast cells
- ✓ Directed Cell Population Growth
- ✓ No Measurable Difference Between Substrates B and C.

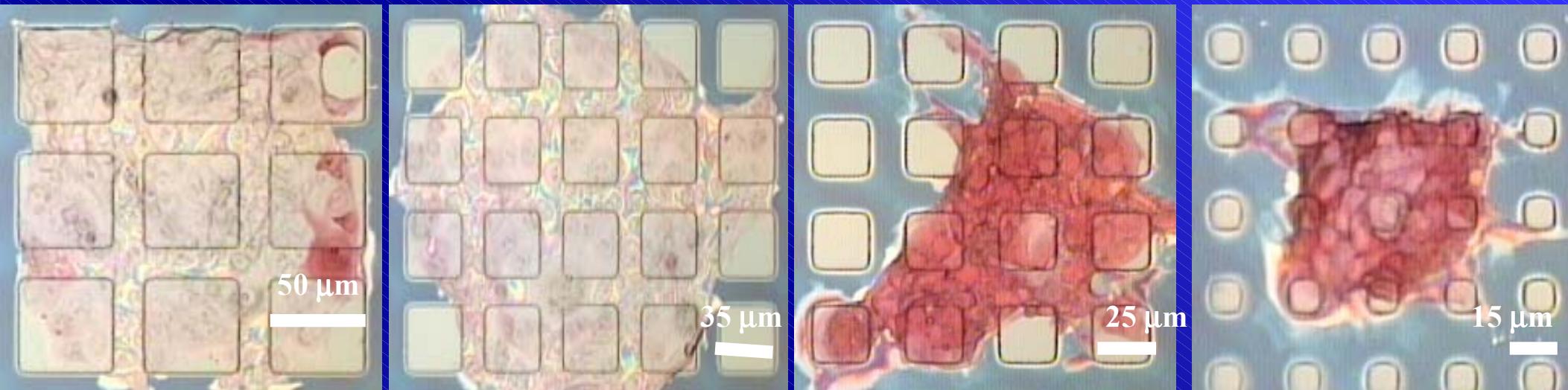


Epithelial cell colony on PLA

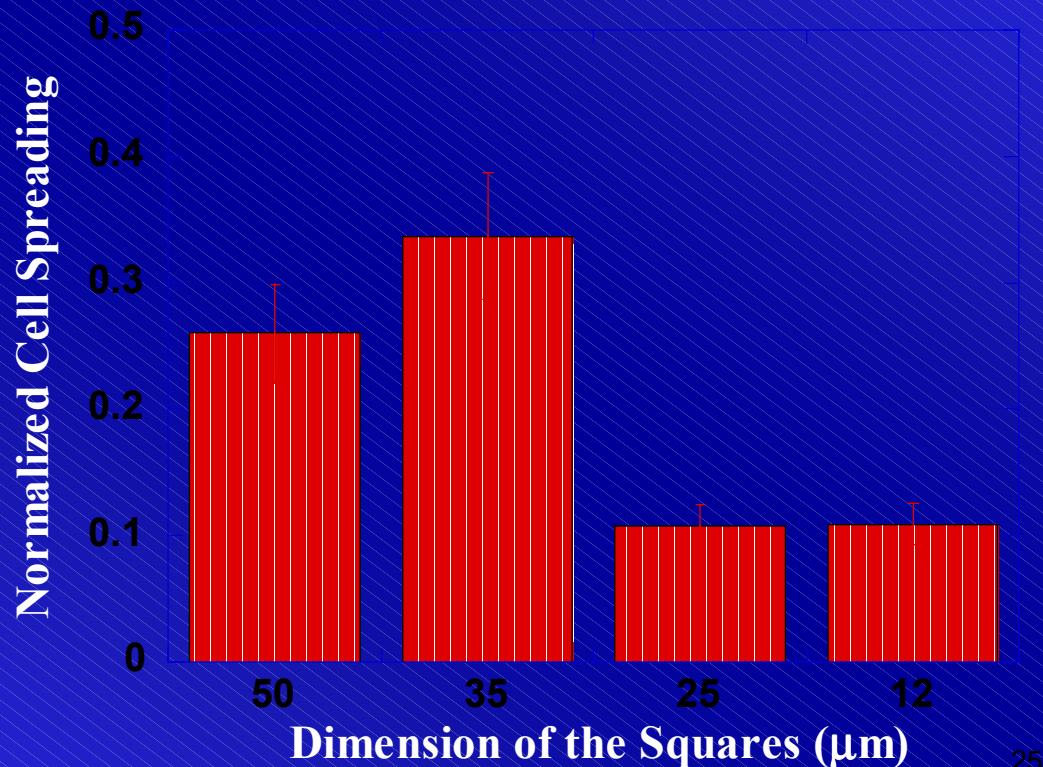


Decreasing Groove Spacing

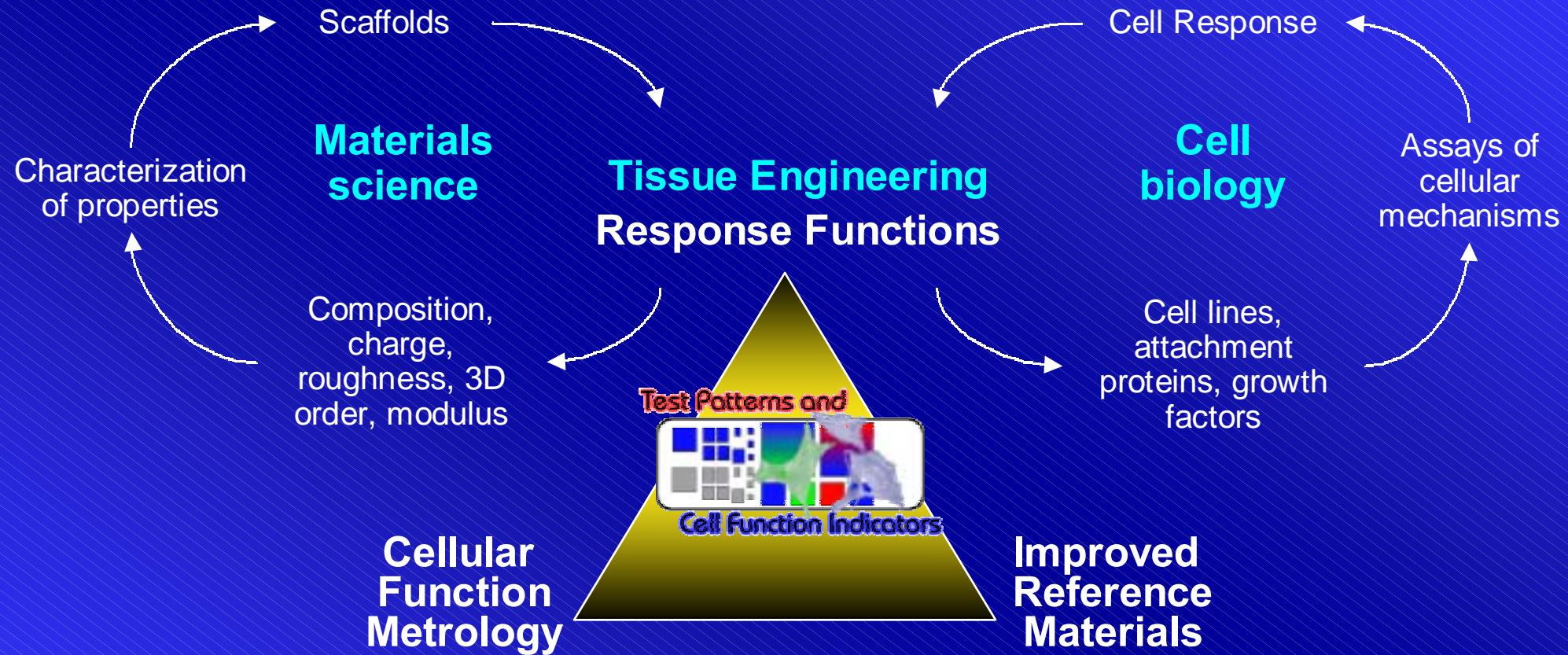
A Closer Look At Square Patterns



- ❖ Darker Membrane Stain is indicative of less cell spreading
- ❖ Small induction of symmetrical growth by pattern
- ❖ Optimal cell proliferation region
- ❖ Cell Proliferate more when the squares are holes

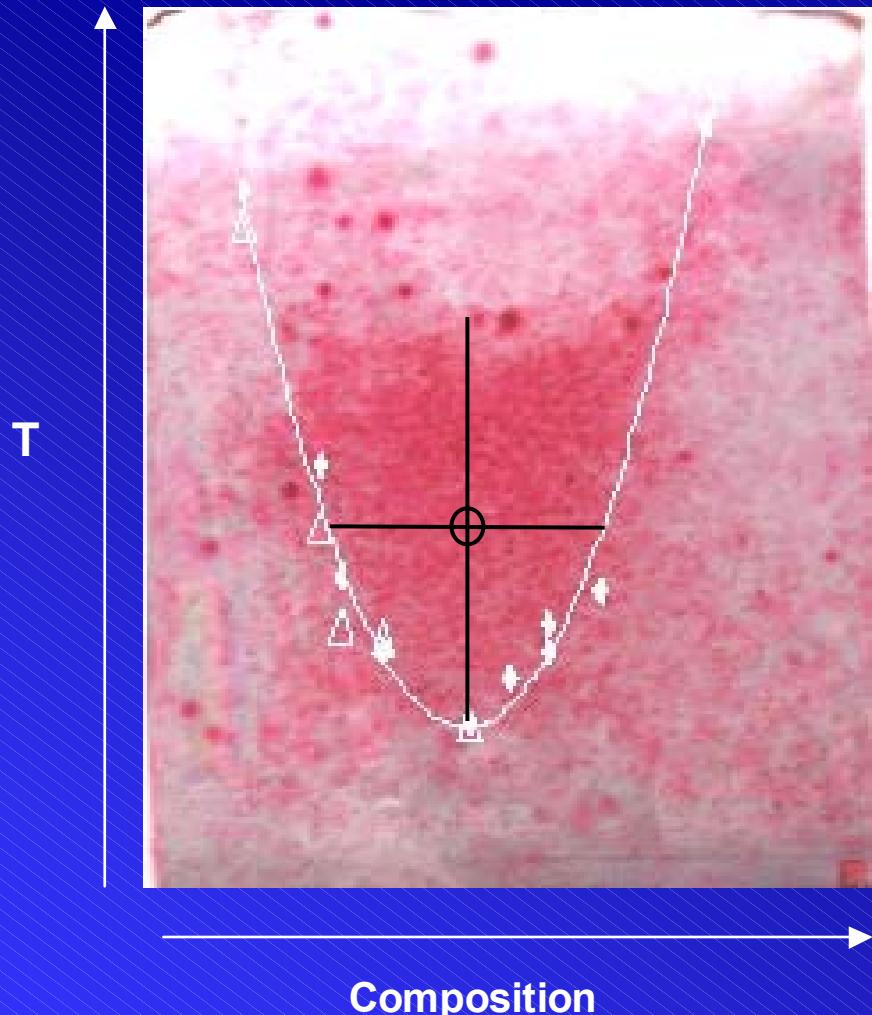


Test Patterns and Cell Function Indicators



Couple materials science and cell biology expertise to prepare reference materials of varying surface properties, cells that signal their functional state, and maps of cell responses

Manufacturability

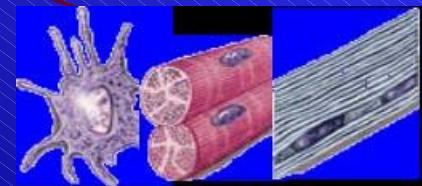


Defining, or at least describing, the tolerance limits for manufacturing materials for biomaterial (e.g. tissue engineering or drug delivery) applications

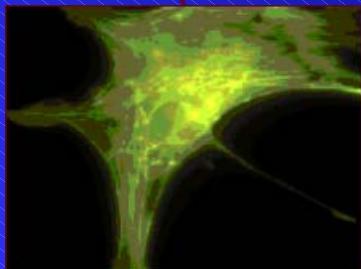
Quality assurance

Regularity qualification

Addressing Regulatory Qualification

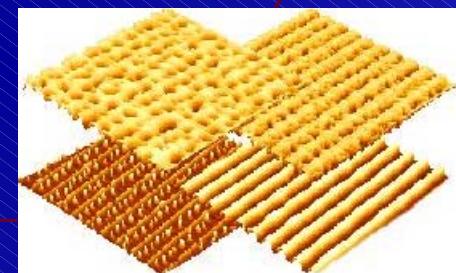


Verification of Scaffold
Functionality



Reference cells

Verification of
Cell Viability



Calibration

Reference materials

Test patterns and cell function indicators constitute a self-contained testing methodology for tissue engineered medical products

Acknowledgements

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